2255-Pos Board B274
Activation of Voltage-Sensitive Phosphatases with Pharmacological and Optogenetic Methods
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Voltage-sensitivephosphatases (VSPs) are proteins that dephosphorylate phosphoinositides (PIs) upon membrane voltage dependent activation. Particularly, Ci-VSP (*Ciona intestinalis* Voltage-Sensitive Phosphatase) is a 5-phosphatase of $PI(4,5)P_2$ and $PI(3,4,5)P_3$ and has so far been studied by electrophysiological means.
Here we developed more broadly applicable methods of membrane depolarization and therefore phosphatase activation. First, depolarization was achieved by coexpression of the K^+ channels TASK and KCNQ and elevation of extracellular potassium concentration. Second, we utilized the capsaicin-activated TRPV1 channel, and last the photosensitive Channel Rhodopsin, ChR2, in order to activate Ci-VSP. Cultured cell lines were transfected with the different channels, Ci-VSP and sensors for PIs, e.g. the tubby and the PLCδ1-PH domain, each tagged with a fluorescent protein. Confocal microscopy was used to visualize and quantify the translocation of the PI-sensors from the membrane to the cytoplasm after the dephosphorylation of PIs. All three methods resulted in successful activation of Ci-VSP, with TRPV1 and ChR2 mediated depolarization to give the most robust results. Specifically, the ChR2 activation provides a very rapid response without the need of solution exchanges, simplifying the experimental procedures. To conclude, we suggest a series of methods that allow the manipulation of PI levels as well as the study of VSPs in living cells without electrophysiological instrumentation.
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Ion Induced Changes in Phosphoinositide Monolayers at Phisiological Concentrations
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Phosphoinositides (PIPs) play a crucial role in many cellular process that occur at the plasma membrane such as calcium release, exocytosis or endocytosis. In order to specifically regulate these functions PIPs must segregate in pools at the plasma membrane. A possible mechanism that could induce and regulate such organization of phosphoinositides is their interaction with bivalent cations. Understanding the physicochemical mechanism that can regulate membrane structure is a crucial step in the development of adaptive biomimetic membrane systems. Using Langmuir monolayers, we investigated the effect of calcium and magnesium on the surface pressure-area/lipid isotherm of monolayer of phosphatidylinositol (PI), phosphatidylinositol bisphosphate (PIP2), dioleoylphosphatidylglycerol (DOPG) and palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). It is found that the decrease of area per lipid, i.e. the increase in aggregation, is mostly dependent on the lipid's head group charge but ion specific. In addition, we discuss changes in free energy and compressibility these monolayer-ion systems.

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Coupling between the N- and C- Termini of Kir2.1 is Critical for Cholesterol Modulation
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In recent years, cholesterol has been emerging as a major regulator of ion channel function. The most common effect of cholesterol on ion channels is a decrease in channel activity that may include a decrease in the open probability, in the unitary conductance and/or in the number of active channels in the membrane. Yet, the mechanism by which cholesterol affects channel function is unclear.
Here we focus on Kir2 channels, a subfamily of constitutively active, strongly inwardly rectifying K^+ channels that set the resting membrane potential and modulate membrane excitability.
We have previously shown that the function of Kir2 channels is suppressed by the elevation of membrane cholesterol and enhanced by cholesterol depletion. Furthermore, our earlier studies have shown that cholesterol sensitivity of Kir2 channels critically depends on a group of residues that form a belt-like structure around the cytosolic pore of the channel in proximity to the transmembrane domain. Surprisingly, however, each of the mutations of the cholesterol sensitivity belt residues that abrogated the cholesterol sensitivity of Kir2.1 also converted these residues to the corresponding residue in Kir2.2. This was completely unexpected because cholesterol sensitivities of Kir2.1 and Kir2.2 channels are very similar.
In this study, we used this phenomenon to gain further insights into cholesterol sensitivity of Kir2 channels. Focusing on the L222I mutation, we screened the differences in the cytosolic domain between Kir2.1 and Kir2.2. Our analysis led to the identification of residues in the N-terminus and in the EF and GA loops of the C-terminus that reversed the effect of the L222I mutation on the cholesterol sensitivity of Kir2.1. These results indicate that an allosteric coupling between the N- and C- termini plays a critical role in cholesterol modulation of Kir2 channels.

2258-Pos Board B277
Analyzing the Significance of the TI/ET Motif for the Substrate Specificity of Phosphoinositide Phosphatases, PTEN and VSP
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The voltage sensitive phosphoinositide (PI) phosphatases (VSPs) and the PI-phosphatase PTEN are members of the PTP phosphatase superfamily, sharing their CX_5R signature motif. PTEN and VSPs differ in substrate specificity, PTEN being a $PI(3,4)P_2/PI(3,4,5)P_3$-3-phosphatase, whereas VSPs are $PI(4,5)P_2/PI(3,4,5)P_3$-5-phosphatases. The most striking difference between both classes of phosphatases lies in the CX5R motif itself, were an alanine in PTEN is replaced by a glycine in VSP. But this amino acid exchange is insufficient to fully explain the difference in specificity (Leitner et al., Biophysical Journal 102(3) p. 246a). Another notable difference between PTEN and VSPs is the absence of the TI-loop motif of PTEN in VSPs, where it is replaced by the ET-motif of the ''gating-loop'' (Liu, Kohout et al., NSMB 2012).
We set out to elucidate the significance of the TI/ET motif, using a set of voltage sensitive phosphatases: Ci-VSP and two engineered VSPs, $PTEN_{CiV}$ and $hVSP1_{CiV}$. The latter contain Ci-VSP's voltage sensing domain and the phosphatase domain of PTEN or hVSP1 (= TPTE2). The effects of TI/ET (in PTEN) or ET/TI (in VSPs) mutations were studied using fluorescent PI sensor domains for the detection of phosphatase activity.
We find that the TI/ET mutation transforms PTEN into a $PI(3,4,5)P_3$-5-phosphatase with no detectable $PI(4,5)P_2$-phosphatase activity. An additional A/G -mutation in the CX_5R-motif is required to induce $PI(4,5)P_2$-5-phosphatase activity, thereby converting PTEN's phosphatase phenotype into that of VSPs. For VSPs the situation appears to be more complex, as we failed to convert their phenotype from 5- to 3-phosphatase by mirroring these mutations (i.e. ET/TI and G/A).
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Ryanodine & IP3 Receptors II

2259-Pos Board B278
Overexpression of RyR1 Enhances Ca^{2+}-Induced Mitochondrial ATP Production in Cardiac H9C2 Cells
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Background: Cytosolic Ca^{2+} elevation is an important trigger for both mitochondrial dynamics and ATP generation in various cell types including cardiac myocytes. Mitochondrial Ca^{2+} influx is mainly mediated by the mitochondrial Ca^{2+} uniporter (MCU). Recent studies have identified additional Ca^{2+} uptake pathways, which exhibit different biophysical properties from MCU including skeletal-muscle type ryanodine receptor isoform1 (RyR1). However it is still unclear that which mitochondrial Ca^{2+} influx mechanism mainly participates in the regulation of mitochondrial morphology/dynamics and energetic functions in cardiac myocytes. **Aim:**To investigate the role of mitochondrial RyR1 in the regulation of mitochondrial morphology and function in cardiac cells. **Methods:**GFP- or non-tagged RyR1 was transiently or stably overexpressed in cardiac H9C2 cells. MCU and RyR2 are also expressed for comparisons. Mitochondrial localization of over-expressed RyR1 was observed by co-expression of mitochondrial matrix-targeted RFP (mtRFP) using confocal microscope and also quantified by Western blotting using mitochondria fractionation. Mitochondrial morphology was evaluated by the calculation of aspect ratio and form factor from mtRFP pictures. Mitochondrial Ca^{2+} concentration ($[Ca^{2+}]_m$) and ATP ($[ATP]_m$) was measured by mitochondrial matrix-targeted Ca^{2+}-sensitive inverse pericam (Mitycam) and FRET-based indicators